The Cellular Mechanism of Glucocorticoid Acceleration of Fetal Lung Maturation

FIBROBLAST-PNEUMONOCYTE FACTOR STIMULATES CHOLINE-PHOSPHATE CYTIDYLTRANSFERASE ACTIVITY

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The cellular mechanism by which glucocorticoids stimulate phosphatidylcholine biosynthesis has been studied in the fetal rat lung in vivo and in cultured fetal rat lung cells of varying levels of complexity. Administration of dexamethasone to pregnant rats at 18 days gestation resulted in a significant increase in saturated phosphatidylcholine content in fetal lung 24 h after injection. Dexamethasone administration increased the activity of fetal lung choline-phosphate cytidyltransferase by 54%. It had no effect on the activities of fetal lung choline kinase and choline phosphotransferase. Exposure of fetal lung type II cells in organotypic cultures (which contain both type II cells and fibroblasts) to cortisol resulted in a 1.6-fold increase in the incorporation of [Me-3H]choline into saturated phosphatidylcholine. The activities of the enzymes in the choline pathway for the de novo biosynthesis of phosphatidylcholine were not significantly altered except for a 105% increase in choline-phosphate cytidyltransferase activity. Treatment of monolayer cultures of fetal type II cells with cortisol-conditioned medium from fetal lung fibroblasts resulted in a 1.5-fold increase in saturated phosphatidylcholine production. This effect correlated with a doubling of choline-phosphate cytidyltransferase activity. Additional evidence that this stimulatory action is mediated by fibroblast-pneumocyte factor, produced by fetal lung fibroblasts in response to cortisol, was obtained. The factor was partially purified from cortisol-conditioned medium of fetal lung fibroblasts by gel filtration and affinity chromatography. Based on biological activity, a 3000-fold purification was obtained. Stimulation of saturated phosphatidylcholine synthesis in type II cells by fibroblast-pneumocyte factor was maximal within 60 min of incubation. Pulse-chase experiments indicated that the stimulatory effect was correlated with an increased conversion of choline phosphate into CDP choline. Moreover, the enhanced phosphatidylcholine formation by fetal type II cells in response to fibroblast-pneumocyte factor was accompanied by decreased levels of cellular choline phosphate.

These findings further support the concept that glucocorticoid action on surfactant-associated phosphatidylcholine synthesis occurs ultimately at the level of the alveolar type II cell and involves fibroblast-pneumocyte factor which stimulates the activity of choline-phosphate cytidyltransferase.

Pulmonary surfactant prevents alveolar collapse and transudation of fluid at low lung volumes by diminishing the surface tension at alveolar air-liquid interfaces (1). The ability of pulmonary surfactant to reduce the surface tension at expiration is of critical importance at birth. Surfactant deficiency is considered to be responsible for the occurrence of respiratory distress syndrome in the premature newborn (2). At the end of gestation lung maturation, especially the onset of surfactant production appears to be regulated by endogenous fetal glucocorticoids (3, 4). Although studies have demonstrated that glucocorticoids enhance the synthesis of surfactant-associated phosphatidylcholine in fetal lung (5–9), the mode of its action remains incompletely understood. In a number of species, glucocorticoids have been shown to stimulate fetal lung choline-phosphate cytidyltransferase activity (5, 6, 8, 10, 11). This enzyme catalyzes the rate-limiting reaction in the formation of phosphatidylcholine in whole lung and in alveolar type II cells, the producers of surfactant (12–14). Hence this enzyme is an attractive candidate for hormonal control. In the present study we have investigated in detail the cellular mechanism by which glucocorticoids stimulate the activity of choline-phosphate cytidyltransferase in the fetal lung.

EXPERIMENTAL PROCEDURES

Materials—Pregnant Sprague-Dawley rats of known gestational age were purchased from Charles River Laboratories, Wilmington, MA. [Me-3H]Choline chloride (specific activity, 80 Ci/mmol) was bought from New England Nuclear. Trypsin (VMP) and collagenase were from Worthington. Gelfoam pads were from The Upjohn Co. and plasticware from Falcon Plastics (Becton Dickinson Co., Oxnard, CA). Medium and sera were obtained from Flow Laboratories, Inc., McLean, VA. Hydrocortisone was purchased from Steraloids, Inc., Wilton, NH and dexamethasone from Schering, Bloomfield, NJ. Fibroblast Cultures—Fibroblasts were isolated from 19-day fetal rat lungs as previously described (15). Briefly, the lungs were dissected from the fetuses and minced into 1-mm³ pieces. The minced tissue was trypsinized in calcium- and magnesium-free Hanks' balanced salt solution containing 0.05% trypsin and 10 µg/ml DNase. Single cells were obtained by filtering the resulting suspension through a 50-µm Nitex filter. The cells were collected by centrifugation and resuspended in minimal essential medium containing 50 µg/ml gentamicin, 2.5 µg/ml Fungizone, and 10% fetal calf serum (MEM)/10%

The abbreviations used are: MEM, minimal essential medium; FCS, fetal calf serum; CS, charcoal-stripped calf serum.

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Terminated after 20 h by liberating the type I1 cells from the organotypic cultures and of fetal lung cells, as noted below. The adherent cells, predominantly fibroblasts (15, 16), were grown to confluence in MEM supplemented with 10% charcoal-stripped calf serum (MEM/10% CS-). At confluence, the cultured fibroblasts were liberated from the Gelfoam sponges with 0.1% collagenase as described by Sevastian et al. (18). The type I1 cells were resuspended in MEM/10% CS- and propagated as monolayer cultures. Yield, purity, and viability were in the same range as previously described (16).

Organotypic Cultures—The sonadherent cells were pelleted by centrifugation, incubated as such for 1 h at 37 °C to allow ressurgulation (16), and then resuspended in MEM containing 10% fetal calf serum. An aliquot (100 μl) of the cell suspension, containing 5–10 x 10^6 cells, was seeded on 1-cm² Gelfoam pads and incubated overnight in Petri dishes at 37 °C in 5% CO₂ in air. The Gelfoam pads were then transferred to new Petri dishes (10 pads/dish) and maintained in MEM/2% CS-.

Type II Cell Cultures—After 3 days of culture, the type II cells were liberated from the Gelfoam sponges with 0.1% collagenase as described by Sevastian et al. (18). The type II cells were resuspended in MEM/10% CS- and propagated as monolayer cultures. Yield, purity, and viability were in the same range as previously described (16).

Measurement of Enzyme Activities—On day 18 of gestation, pregnant rats were laparotomized intraperitoneally with dexamethasone (0.2 mg/kg) in dimethyl sulfoxide. Controls were similarly injected with vehicle alone. Approximately 24 h after the injections, the fetuses were delivered by hysterotomy, put to death, and the lungs isolated. The freshly excised lungs were homogenized with a Dounce homogenizer in 0.145 M NaCl, 50 mM Tris-HCl (pH 7.4), 50 mM NaF, and 0.5 mM EDTA. The homogenate was stored at -20 °C until used for enzyme assays. The remainder was extracted for lipid determination.

Organotypic cultures of fetal lung cells were incubated in serum-free MEM with and without 100 nM cortisol. The incubations were terminated after 20 h by liberating the type II cells from the organotypic cultures with 0.1% collagenase (18). The type II cells were washed with Tris saline, pelleted, resuspended in Tris saline, and homogenized with a Dounce homogenizer. The homogenate was stored at -20 °C until enzyme activities were measured.

Type II cells in primary culture were incubated in serum-free MEM in the presence of either 100 nM cortisol or of cortisol-conditioned medium from fetal lung fibroblasts (17). After 20 h of incubation, the medium was removed and the cells were washed with Tris saline. The cells were scraped from the dishes in Tris saline and subsequently homogenized, as above. The homogenate was stored at -20 °C until use.

Choline kinase activity was assayed by measuring the rate of incorporation of radioactive choline into choline phosphate. The incubation medium (0.1 ml) contained 100 mM Tris-HCl (pH 8.0), 30 mM MgCl₂, 10 mM ATP, 0.25 mM [Me-³H]choline (specific activity, 50 μCi/μmol), and up to 100 μg of protein. After 15 min of incubation at 37 °C, the reaction was stopped by the addition of 30 μl of glacial acetic acid. The reaction product, choline phosphate, was separated from radioactive choline by paper chromatography as described previously (19).

Choline phosphotransferase activity was assayed by measuring the rate of incorporation of labeled CDP choline into phosphatidylcholine using endogenous liver lipids as the acceptor substrate. The homogenate (50–100 μg of protein) was incubated at 37 °C in 0.18 ml of incubation medium containing 100 mM Tris maleate (pH 7.4), 25 mM MgCl₂, 10 mM β-mercaptoethanol, 1 mM EDTA, and 0.5 mM CDP [Me-³H]choline (specific activity, 42 Ci/mmol). The formation of radioactive phosphatidylcholine was measured as follows. Fetal lung type I1 cells in primary culture were incubated in serum-free MEM with 100 μCi/ml [Me-³H]choline at 37 °C. After 5 h of incubation, the radioactive medium was removed and the cells were rinsed 3 times with cold Hanks’ balanced salt solution to remove unincorporated labeled choline. Immediately afterward, the chase medium (MEM) (with or without affinity-purified fibroblast-pneumocyte factor) was added to the cells. After various times up to 2 h, the incubation was terminated by removal of the medium and addition of ice-cold methanol/H₂O (5:4, v/v). The cells were scraped from the dishes, and the radioactivity incorporated into choline-containing compounds was determined.

**Choline Kinase Activity**—One liter of cortisol-conditioned serum-free medium from 19-day rat fetal lung fibroblasts was partially desalted and concentrated in a stirred stream across a membrane with molecular mass cutoff of 2,000 daltons (Amicon). The concentrate was lyophilized, suspended in 1 M acetic acid, and fractionated on a Bio-Gel P-30 column as described previously (22). Column fractions with approximate molecular mass of 20,000–4,000 daltons were pooled, lyophilized, and suspended in 1.0 M HCl followed by neutralization with an equal volume of 1.0 M NaOH. The resuspended proteins were extensively dialyzed against phosphate-buffered saline and applied to an affinity column prepared with monoclonal antibodies derived from hybridomas grown in mouse peritoneum (23). The antibodies were purified from ascites fluid by 50% ammonium sulfate precipitation followed by gel filtration on Sephacryl S-300 using 0.5 M NaCl, 50 mM Tris-HCl (pH 7.5) as eluent. Fractions rich in IgM were coupled to CNBr-activated Sepharose 4B (Pharmacia). The resuspended proteins were applied to a 10-cm column of immunoabsorbent equilibrated with phosphate-buffered saline. The immunoabsorbent was then washed with 10 column volumes of phosphate-buffered saline, and subsequently specific proteins bound to the column were eluted with 0.1 M glycine HCl (pH 2.5–2.8), neutralized with 1 M Tris, and tested for activity in a previously described bicarbonate buffer.

**Time Course Experiment**—The effect of affinity-purified fibroblast-pneumocyte factor on the formation of saturated phosphatidylcholine was measured as follows. Fetal lung type II cells in primary culture were incubated in serum-free MEM in the presence or absence of fibroblast-pneumocyte factor. The incubation medium was supplemented with 1 μCi/ml [Me-³H]choline. After the desired incubation times, the medium was removed and the cells were recovered by trypsinization. An aliquot of the cell suspension was counted electronically, and the lipids were extracted from the cell suspension.

**Pulse-Chase Experiments**—Monolayer cultures of fetal type II cells were washed free of serum and preincubated for 60 min in serum-free MEM. After this period, the cells were washed with 5 μCi/ml [Me-³H]choline. At the end of the 60-min pulse period, the radioactive medium was removed and the cells were rinsed 3 times with cold Hanks’ balanced salt solution to remove unincorporated labeled choline. Immediately afterward, the chase medium (MEM) (with or without affinity-purified fibroblast-pneumocyte factor) was added to the cells. After various times up to 2 h, the incubation was terminated by removal of the medium and addition of ice-cold methanol/H₂O (5:4, v/v). The cells were scraped from the dishes, and the radioactivity incorporated into choline-containing compounds was determined.

**Continuous Labeling Experiments**—Fetal type II cells in primary culture were washed free of serum and incubated in serum-free medium containing 1 μCi/ml [Me-³H]choline at 37 °C. After 5 h of incubation, affinity-purified fibroblast-pneumocyte factor was added, and the incubation was continued for an additional 2 h. The
uptake of labeled choline by the cells and the incorporation into its metabolites were monitored at various times after the addition of fibroblast-pneumonocyte factor. The incubations were terminated by removal of the medium, washing of the cells, and addition of ice-cold methanol/H₂O (6:4, v/v). The cells were scraped from the dishes, and the incorporation of radioactivity into choline-containing compounds was determined.

Analysis of Lипids—Lipids were extracted from fetal lung homogenate and cell suspensions by the method of Bligh and Dyer (21). Phosphatidylcholine was isolated from the lipid extract by thin-layer chromatography on Silica Gel G plates with chloroform/methanol/H₂O (65:25:4, v/v) as eluent. For saturated phosphatidylcholine, the lipid extracts were treated with osmium tetroxide prior to chromatography on Silica Gel H plates with chloroform/methanol/H₂O (65:25:4, v/v) as eluent (24). The phosphatidylcholine spots were visualized with bromothymol blue and scraped into either test tubes for the determination of lipid content or into scintillation vials for radioactivity measurement.

Analysis of Choline-containing Compounds—The aqueous phase remaining after lipid extraction was evaporated to dryness and the residue dissolved in water. Choline, choline phosphate, and CDP choline were separated by thin-layer chromatography on Silica Gel H plates with chloroform/methanol/concentrated NH₄OH (65:50:5, v/v) as developing solvent. After visualization with iodine vapor, choline and its metabolites were scraped into scintillation vials and assayed for radioactivity.

Other Methods—Protein was determined by the method of Bradford (25) with bovine serum albumin as standard. The phosphorus content of the saturated phosphatidylcholine in fetal lung was determined according to the method of Bartlett (26).

RESULTS

Animal Studies—In agreement with previous studies (5–9), maternal administration of dexamethasone to pregnant rats significantly increased the saturated phosphatidylcholine content in lung tissue of the fetuses (56.9 ± 6.4 versus 33.8 ± 2.4 nmol/mg protein, dexamethasone versus control, mean ± S.E. (n = 5), p < 0.05). The activities of the enzymes catalyzing the individual steps of de novo phosphatidylcholine synthesis from control and dexamethasone-treated lungs were measured to correlate the increase in saturated phosphatidylcholine content with activities of the relevant enzymes. Table I demonstrates that choline kinase and choline phosphotransferase activities are unchanged by the dexamethasone treatment. However, choline-phosphate cytidylyltransferase activity is significantly increased. Similar stimulatory effects of glucocorticoids on choline-phosphate cytidylyltransferase activity in fetal lung have been reported earlier (5, 6, 8, 10, 11).

Organotypic Culture Studies—Results derived from studies carried out with whole lung cannot be directly extrapolated to surfactant-producing type II cells. Therefore, it seemed of interest to study the glucocorticoid effect on saturated phosphatidylcholine synthesis in organotypic cultures of fetal lung cells. Cortisol (100 nM) has previously been shown to stimulate the incorporation of [Me-³H]choline into saturated phosphatidylcholine of type II cells in organotypic cultures (23).

In the present study, we found that the cortisol-stimulated synthesis of saturated phosphatidylcholine (as measured by choline incorporation) in type II cells in organotypic cultures (8492 ± 1100 versus 5169 ± 860 dpm/10⁶ cells, cortisol versus control, mean ± S.E. (n = 9), p < 0.05) correlated with an increased activity of choline-phosphate cytidylyltransferase (Table II). The activities of choline kinase and choline phosphotransferase are not significantly changed after exposure of organotypic cultures to cortisol. Previous studies from our laboratory have demonstrated that antibodies raised against fibroblast-pneumonocyte factor block this cortisol-stimulated saturated phosphatidylcholine production by type II cells in organotypic cultures (23). This result led to the conclusion that the stimulatory effect of cortisol on surfactant production in this heterogeneous cell system is mediated by fibroblast-pneumonocyte factor. This previous observation in conjunction with the present study strongly indicates that the observed increase in choline-phosphate cytidylyltransferase activity in type II cells after exposure of organotypic cultures to cortisol is a result of the fibroblast-pneumonocyte factor action on type II cells.

Type II Cell Studies—Since cortisol-conditioned medium of fetal lung fibroblasts stimulates [Me-³H]choline incorporation into saturated phosphatidylcholine of type II cells (7393 ± 488 versus 5068 ± 468 dpm/10⁶ cells, cortisol-conditioned medium versus control, mean ± S.E. (n = 9), p < 0.01) we examined the effect of choline-phosphate cytidylyltransferase activity. Table III indicates that the cortisol-conditioned medium increases the activity of the enzyme in type II cells. Choline kinase and choline phosphotransferase activities are not changed in these cells (data not shown). Although this observation suggests that fibroblast-pneumonocyte factor specifically stimulates choline-phosphate cytidylyltransferase activity in type II cells, the role of other possible effectors in the medium has not been totally excluded. To circumvent this consideration, we purified fibroblast-pneumonocyte factor from conditioned medium and examined its effect on phosphatidylcholine metabolism in type II cells.

Purification of Fibroblast-Pneumonocyte Factor—Fibroblast-pneumonocyte factor was purified from cortisol-conditioned medium of fetal lung fibroblasts by gel filtration and affinity chromatography. The small quantities present in the media were not sufficient for monitoring the efficacy of the purification by standard gel electrophoresis techniques. However, gel electrophoresis did reveal that the material was not purified to homogeneity. The biologic activity of the material at various stages of the purification was determined and the effectiveness of the purification scheme expressed in terms of specific activity (Table IV). It can be seen that a 3000-fold purification in bioactivity was obtained. It can be noted that total activity doubled after gel filtration. Previous studies (28) have shown that fetal lung fibroblasts also elaborate an inhibitory activity of type II cells. Therefore, it seemed of interest to study the glucocorticoid effect on saturated phosphatidylcholine synthesis in organotypic cultures of fetal lung cells. Cortisol (100 nM) has previously been shown to stimulate the incorporation of [Me-³H]choline into saturated phosphatidylcholine of type II cells in organotypic cultures (23). In the present study, we found that the cortisol-stimulated synthesis of saturated phosphatidylcholine (as measured by choline incorporation) in type II cells in organotypic cultures (8492 ± 1100 versus 5169 ± 860 dpm/10⁶ cells, cortisol versus control, mean ± S.E. (n = 9), p < 0.05) correlated with an increased activity of choline-phosphate cytidylyltransferase (Table II). The activities of choline kinase and choline phosphotransferase are not significantly changed after exposure of organotypic cultures to cortisol. Previous studies from our laboratory have demonstrated that antibodies raised against fibroblast-pneumonocyte factor block this cortisol-stimulated saturated phosphatidylcholine production by type II cells in organotypic cultures (23). This result led to the conclusion that the stimulatory effect of cortisol on surfactant production in this heterogeneous cell system is mediated by fibroblast-pneumonocyte factor. This previous observation in conjunction with the present study strongly indicates that the observed increase in choline-phosphate cytidylyltransferase activity in type II cells after exposure of organotypic cultures to cortisol is a result of the fibroblast-pneumonocyte factor action on type II cells.

**Table I**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Dexamethasone</th>
<th>Treated/ control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/min/mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline kinase</td>
<td>0.49 ± 0.05 (7)</td>
<td>0.51 ± 0.04 (7)</td>
<td>1.04</td>
</tr>
<tr>
<td>Choline-phosphate cytidylyltransferase</td>
<td>1.65 ± 0.13 (7)</td>
<td>2.21 ± 0.13 (7)</td>
<td>1.34</td>
</tr>
<tr>
<td>Choline phosphotransferase</td>
<td>0.077 ± 0.003 (7)</td>
<td>0.069 ± 0.006 (7)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

* NS, not significant.


Regulation of Phosphatidylcholine Synthesis in Fetal Lung

TABLE II

Effect of cortisol on enzyme activities for phosphatidylcholine formation in fetal rat lung type II cells in organotypic cultures

Organotypic cultures were prepared with lung cells from fetal rats at 19 days gestation and incubated with or without cortisol. After 20 h of incubation the type II cells were isolated from the organotypic cultures, and enzyme activities were assayed. The data are presented as means ± S.E. with the number of observations indicated in parentheses. Significance was determined using Student’s t test.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Cortisol</th>
<th>Treated/</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/min/mg protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline kinase</td>
<td>0.71 ± 0.09 (10)</td>
<td>0.91 ± 0.09 (10)</td>
<td>1.28</td>
<td>NS</td>
</tr>
<tr>
<td>Choline phosphate cytidylytransferase</td>
<td>1.88 ± 0.36 (10)</td>
<td>3.84 ± 0.46 (10)</td>
<td>2.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Choline phosphotransferase</td>
<td>0.019 ± 0.002 (10)</td>
<td>0.023 ± 0.002 (10)</td>
<td>1.20</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS, not significant.

TABLE III

Effect of fibroblast-pneumonocyte factor on choline-phosphate cytidylytransferase activity in fetal rat lung type II cells

Fetal type II cells were isolated from organotypic cultures, grown as monolayers, and incubated with either 100 nM cortisol (control condition) or cortisol-conditioned medium of fetal lung fibroblasts. After 20 h of incubation the choline-phosphate cytidylytransferase activity was assayed. The data are presented as means ± S.E. of four experiments each carried out in triplicate. Significance was determined using Student’s t test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific activity nmol/min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.32 ± 0.25</td>
</tr>
<tr>
<td>Cortisol-conditioned medium</td>
<td>2.64 ± 0.43*</td>
</tr>
</tbody>
</table>

*p 0.01.

TABLE IV

Purification of fibroblast-pneumonocyte factor from cortisol-conditioned serum-free medium of fetal rat lung fibroblasts

One liter of fibroblast-conditioned medium (FCM) was purified by gel filtration chromatography and affinity chromatography. The efficiency of the purification scheme is based on determination of the biological activity of the various fractions as tested by bioassay (17).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Specific activity units/g protein</th>
<th>Recovery %</th>
<th>Purification factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCM (1 liter)</td>
<td>32 x 10⁶</td>
<td>0.08</td>
<td>100</td>
</tr>
<tr>
<td>Bio-Gel P60</td>
<td>9.7 x 10⁶</td>
<td>0.65</td>
<td>246</td>
</tr>
<tr>
<td>Affinity</td>
<td>3</td>
<td>263.60</td>
<td>31</td>
</tr>
</tbody>
</table>

*One unit is defined as the amount necessary to give a 10% increase in saturated phosphatidylcholine formation above control in a 60-min incubation.

TABLE V

The effect of fibroblast-pneumonocyte factor on saturated phosphatidylcholine formation in fetal type II cells as a function of the amount of protein

Fetal type II cells were incubated with various amounts of affinity-purified fibroblast-pneumonocyte factor. The incubation medium was supplemented with 1 µCi/ml [Me-³H]choline. After 4 h of incubation the cells were harvested and the [Me-³H]choline incorporated into saturated phosphatidylcholine was measured. The mean ± S.E. of four determinations is presented.

<table>
<thead>
<tr>
<th>FPF amount</th>
<th>SPC synthesis in fetal type II cells</th>
<th>Ratio (treated/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>dpm/10⁶ cells</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2603 ± 145</td>
<td>1.0</td>
</tr>
<tr>
<td>2.5</td>
<td>3013 ± 292</td>
<td>1.16</td>
</tr>
<tr>
<td>25</td>
<td>4201 ± 634</td>
<td>1.61</td>
</tr>
<tr>
<td>250</td>
<td>3988 ± 651</td>
<td>1.54</td>
</tr>
</tbody>
</table>

FIG. 1. The effect of fibroblast-pneumonocyte factor on saturated phosphatidylcholine formation in fetal type II cells as a function of time. Fetal type II cells were incubated with or without 25 ng/ml affinity-purified fibroblast-pneumonocyte factor as described under "Experimental Procedures." The results are expressed as per cent increase in saturated phosphatidylcholine formation above control.

The removal of this inhibitor by the initial gel filtration step suggests that the monoclonal antibody has a low affinity for fibroblast-pneumonocyte factor. Despite the large losses, this step affected the major portion of the purification procedure.

Table V indicates that a maximal response on saturated phosphatidylcholine synthesis by fetal alveolar type II cells was obtained at a concentration of 25 ng/ml affinity-purified fibroblast-pneumonocyte factor. This concentration was used in all subsequent studies.

The time course for the effect of affinity-purified fibroblast-pneumonocyte factor on choline incorporation into saturated phosphatidylcholine by fetal alveolar type II cells is shown in Fig. 1. Maximal activity was observed after 1 h of incubation. This finding supports the concept that the stimulation is due to enzyme activation rather than new enzyme synthesis.

Pulse-Chase and Continuous Labeling Studies—A pulse-chase study (Fig. 2) demonstrated that the presence of affinity-purified fibroblast-pneumocyte factor in the chase medium increases the rate of disappearance of label from choline phosphate and the rate of its appearance in phosphatidylcholine. The radioactivity associated with CDP choline is unchanged during the chase with fibroblast-pneumocyte factor. Furthermore, the decrease in radioactivity associated with choline is not affected by fibroblast-pneumocyte factor. These observations are in line with the idea that the activity of choline-phosphate cytidylyltransferase in fetal type II cells is stimulated by fibroblast-pneumocyte factor.

To obtain further evidence for the stimulatory effect of

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The incorporation of choline into phosphatidylcholine is increased. The enhanced phosphatidylcholine formation is accompanied by a decrease in the choline phosphate level. Pool sizes of choline and CDP choline are not altered by the addition of fibroblast-pneumocyte factor. Thus, the observed increase in choline incorporation into phosphatidylcholine and the concomitant decrease in choline phosphate in the presence of fibroblast-pneumocyte factor again support the concept that fibroblast-pneumocyte factor stimulates choline-phosphate cytidylyltransferase activity in fetal type II cells.

**FIG. 2.** The effect of fibroblast-pneumocyte factor (FPF) on the metabolism of choline in fetal type II cells. Fetal type II cells were pulsed with [Me-3H]choline (5 μCi/ml) for 60 min and subsequently chased in the presence or absence of 25 ng/ml affinity-purified fibroblast-pneumocyte factor. At various times up to 2 h, the cells were harvested, and radioactivity in choline, choline phosphate, and CDP choline was determined. The start of the chase period is indicated as time zero. The data are representative of two experiments, each carried out in quadruplicate.

**FIG. 3.** The effect of the addition of fibroblast-pneumocyte factor (FPF) on the radioactive pool sizes of the choline-containing metabolites in fetal type II cells. Fetal type II cells were incubated with 1 μCi/ml [Me-3H]choline until isotopic equilibrium of the intermediate metabolites was obtained. Hereafter, 25 ng/ml affinity-purified fibroblast-pneumocyte factor was added to the medium, and the incubations were continued. At various times thereafter, the cells were harvested, and radioactivity in choline, choline phosphate, CDP choline, and phosphatidylcholine was determined. The time of addition of fibroblast-pneumocyte factor is indicated as time zero. The data are representative of two experiments, each carried out in quadruplicate.

Although it has been known for a number of years that glucocorticoids stimulate phosphatidylcholine formation in the lung (3, 4), the mechanism of its effect has not been completely delineated. In the present study we have confirmed previous observations that glucocorticoids enhance surfactant-associated phosphatidylcholine production and that this effect is mediated by an activation of choline-phosphate cytidylyltransferase (5, 6, 8, 10, 11). Studies in vivo (23, 28) and in vitro (16, 17, 22) have indicated, however, that the glucocorticoid effect on lung maturation is dependent upon intercellular interaction. In response to glucocorticoid, the fetal lung fibroblast produces a protein, fibroblast-pneumocyte factor (16, 17, 28, 29), which in turn stimulates the formation of saturated phosphatidylcholine, the major surface-active component of pulmonary surfactant, by fetal alveolar type II cells (16, 17, 22). Our present results provide evidence that the stimulatory effect of fibroblast-pneumocyte factor on phosphatidylcholine formation by fetal type II cells is mediated by an increased conversion of choline phosphate into CDP choline, a reaction catalyzed by choline-phosphate cytidylyltransferase. A 2-fold stimulation of cytidylyltransferase could be shown in type II cells in organotypic cultures exposed to cortisol. Moreover, a similar stimulation was observed in primary cultures of fetal type II cells treated with fibroblast-pneumocyte factor. The acceleration of the conversion of choline phosphate into CDP choline was also demonstrated by pulse-chase studies and correlated with enhanced synthesis of phosphatidylcholine. Furthermore, the levels of choline phosphate were decreased in fetal type II cells exposed to fibroblast-pneumocyte factor, which would be expected if fibroblast-pneumocyte factor activates the formation of CDP choline and subsequently that of phosphatidylcholine. Overall, the results strongly indicate that the rate of surfactant phosphatidylcholine biosynthesis is determined by the activity of choline-phosphate cytidylyltransferase.

The mechanism by which fibroblast-pneumocyte factor stimulates the cytidylyltransferase activity is unknown. Several studies have demonstrated that cytidylyltransferase activity is controlled by enzyme-modulator interactions rather than by changes in the number of molecules (30, 31). The rapidity of the fibroblast-pneumocyte factor effect is in keeping with this concept. Growing evidence has been obtained from studies with numerous cell types (32–37) that phosphatidylcholine synthesis is regulated by translocation of inactive choline-phosphate cytidylyltransferase from the cytosol to the endoplasmic reticulum where it is activated (35). A similar model for the regulation of phosphatidylcholine synthesis has been proposed by Weinhold et al. (38) for this enzyme in the lung. Increased phosphatidylcholine synthesis during lung development was shown to be associated with a redistribution of enzyme from cytosol to microsomes in the whole lung (38). In addition, it has been demonstrated that the activity of the microsomal enzyme in rat lung increases
just before birth (39). The mechanism involved in the association and dissociation of choline-phosphate cytidylyltransferase with the endoplasmic reticulum is not known, but preliminary evidence suggests that the translocation may be controlled by either reversible phosphorylation of the enzyme (40, 41) or by direct effects of long chain fatty acids or their CoA derivatives on the enzyme (35, 42, 43). Future studies will be directed toward the mechanism by which fibroblast-pneumonocyte factor modulates the choline-phosphate cytidylyltransferase activity in fetal alveolar type II cells.

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